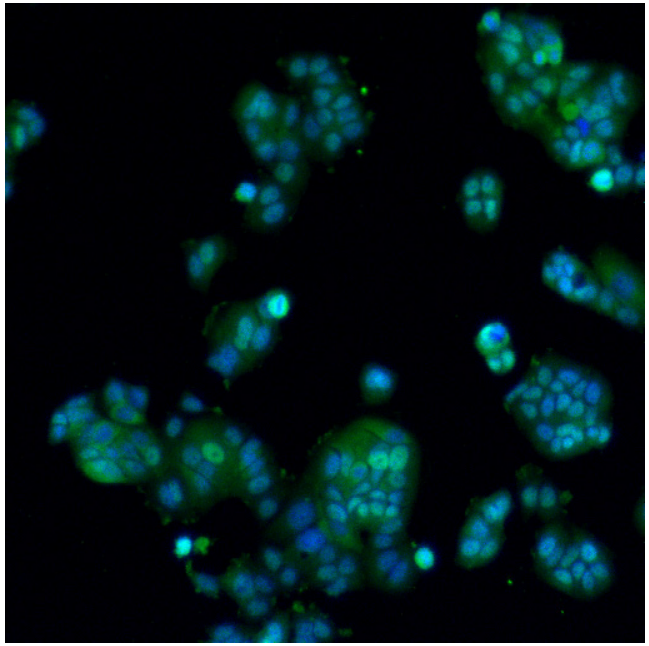
Confocal vs. Widefield Imaging for Cytoplasm to Nucleus Translocation

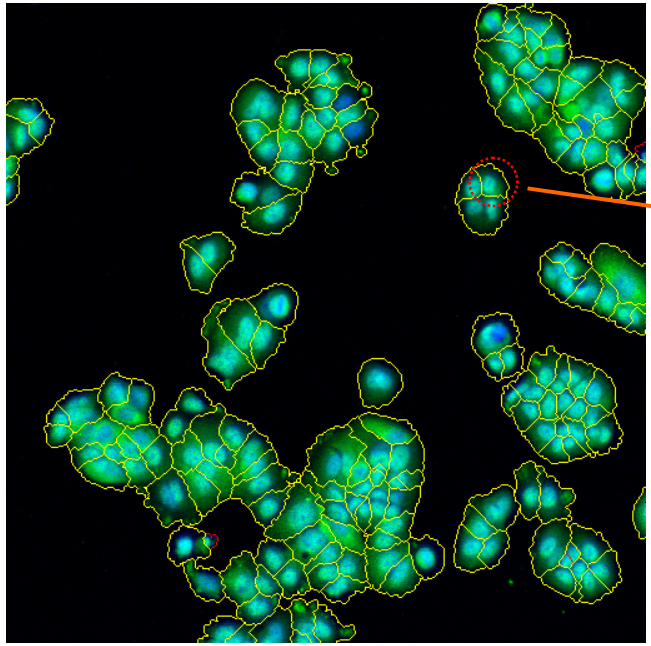
Ilya Ravkin, Vitra Bioscience

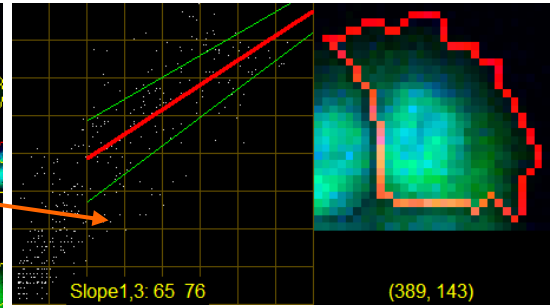
1. Experiment was performed on MCF7 cells to assess translocation of NFκB transcription factor at zero and high concentration of TNFα.
2. Images of the same field were acquired on Nikon TE2000 with Retiga EX camera and with Nikon Confocal Attachment - both with 10X PlanFluor objective.
3. Three classes of CNT measures were calculated: slope-based (Slope1 and Slope3), ring-based (N2R_AW, N2R_AM), nucleus-based (N_AM).
4. Quality was measured by Z-factor applied not as usual to populations of replicas, but to populations of cells in positive and negative states. This gives numerically very low Z values, but presents a more direct comparison of the different measures.
5. N2R_AM is similar to the commonly used measures described in the Cellomics patent 6,671,624. It is based on identifying nuclear mask and taking the ratio of the average intensity of the signal stain in the nucleus to the average intensity of the signal stain in the ring around the nucleus.
6. N2R_AW is similar to N2R_AM, except that it is not based on segmentation, but on weighing the signal image by the nuclear image.
7. N_AM is average intensity of the signal stain in the nucleus (after cell-by-cell intensity normalization).

Positive Images

Confocal



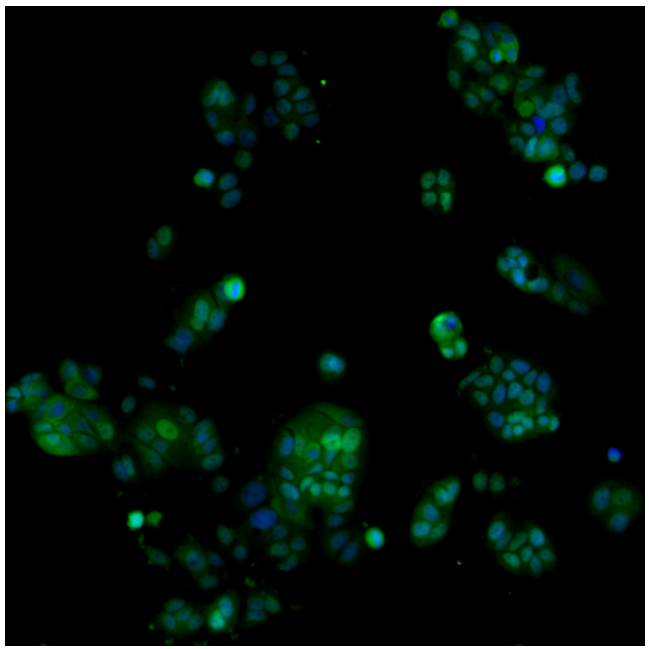


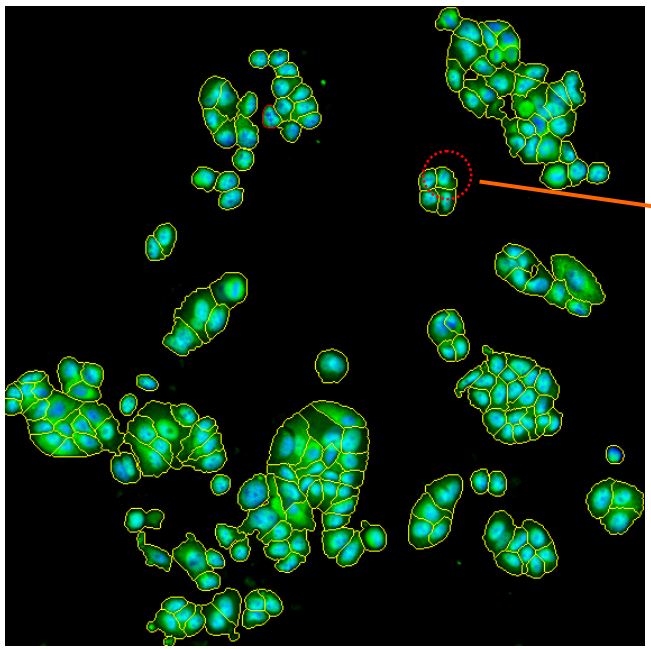


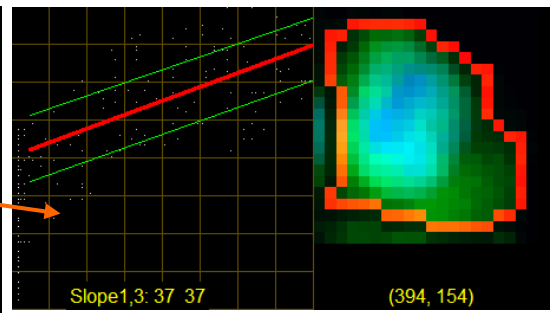
Slope1 = 65,

Slope3 = 76,

Camera





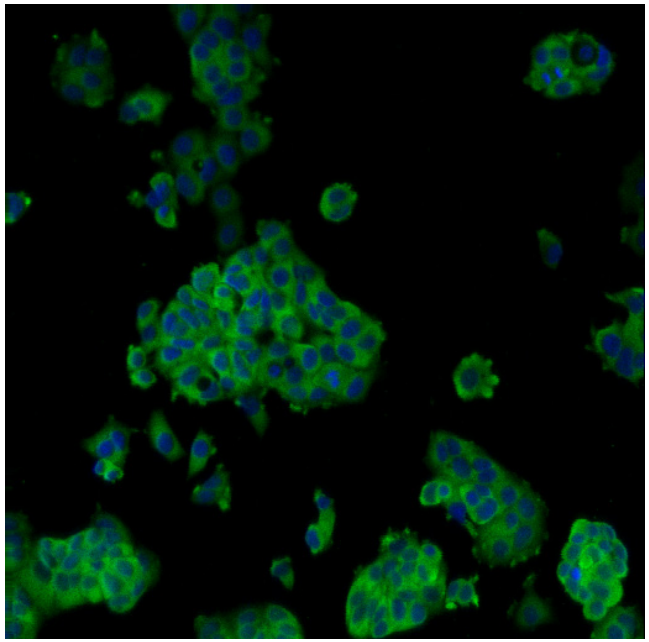


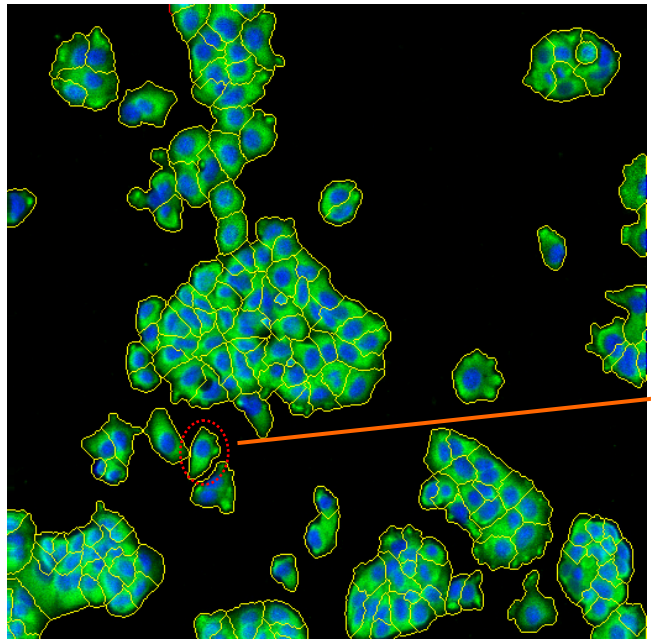
Slope1 = 37,

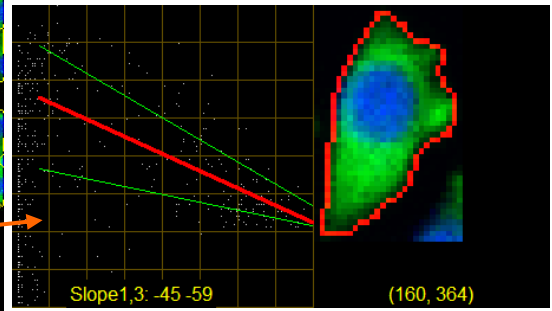
Slope3 = 37,

Negative Images

Confocal



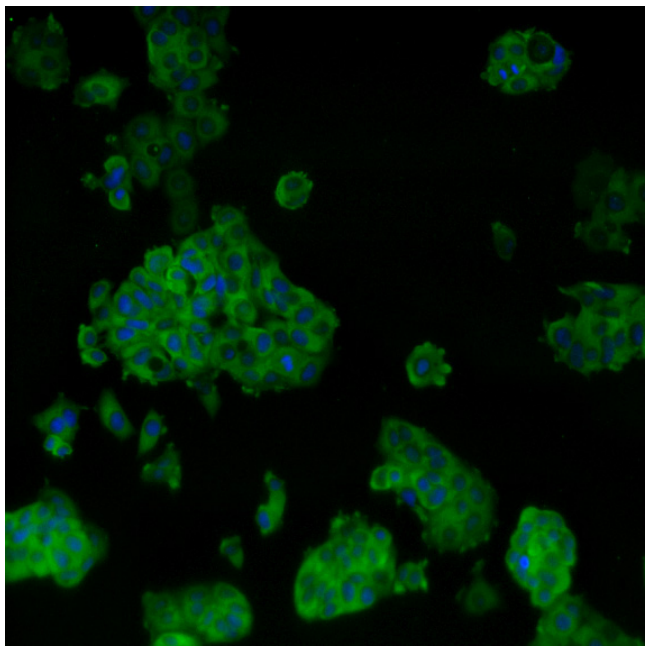


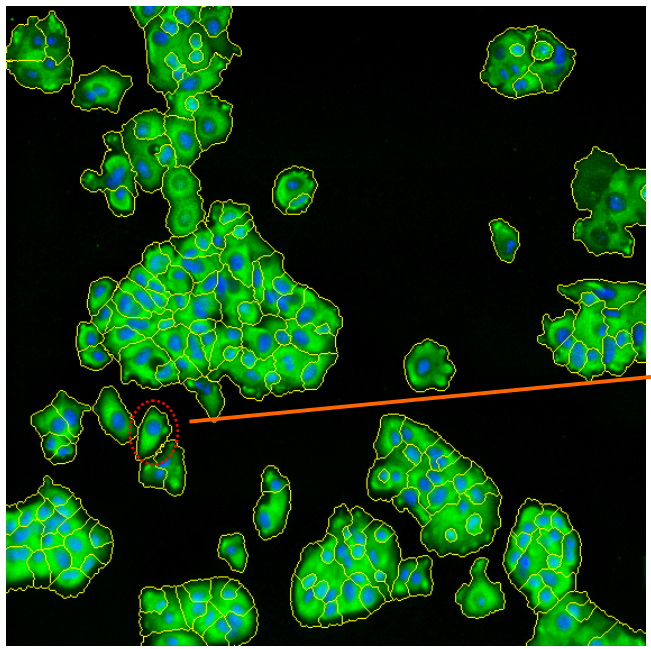


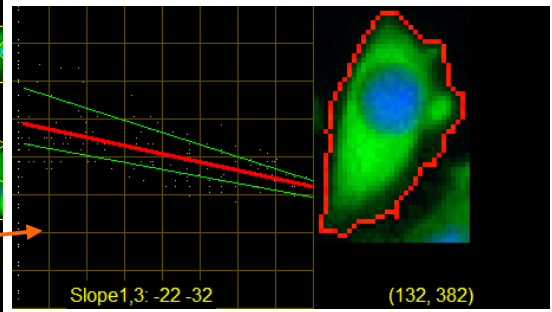
Slope1 = -45,

Slope3 = -59,

Camera







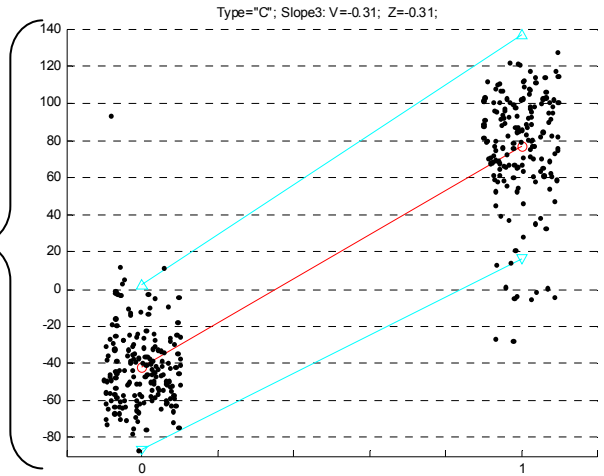
Slope1 = -22,

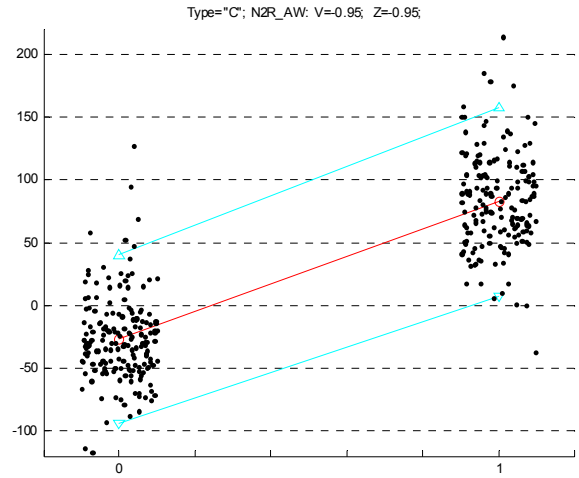
Slope3 = -32,

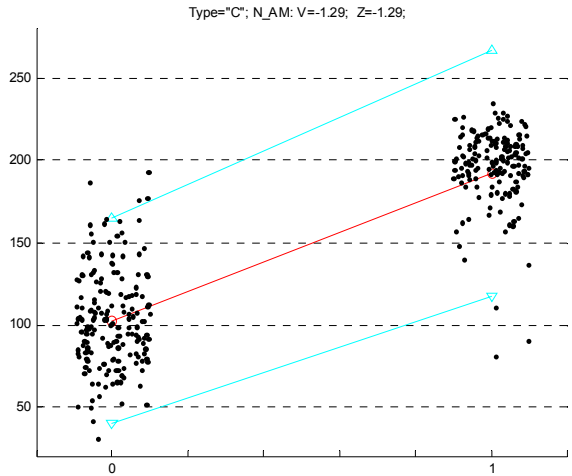
Individual cell analysis

Slope3 | N2R_AW | N_AM

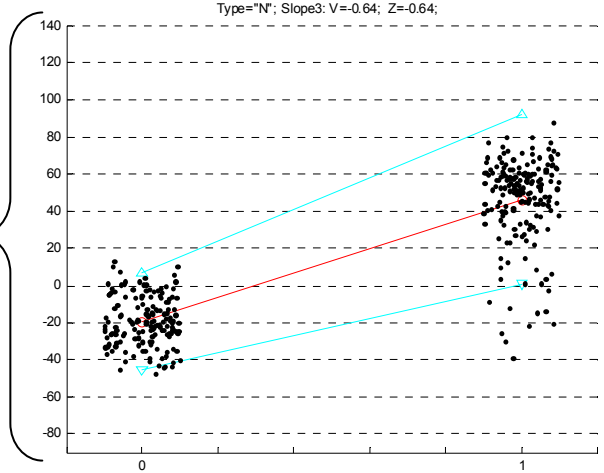
Confocal

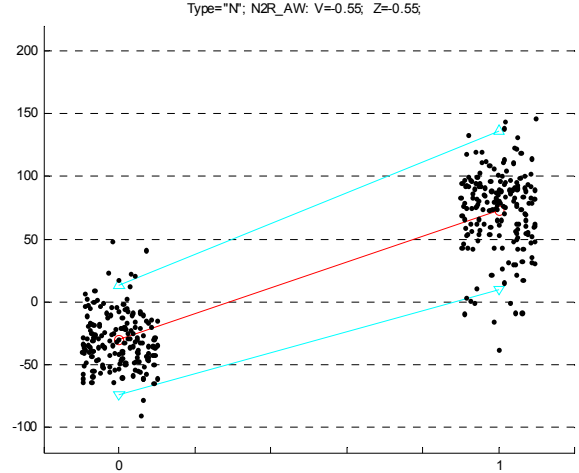


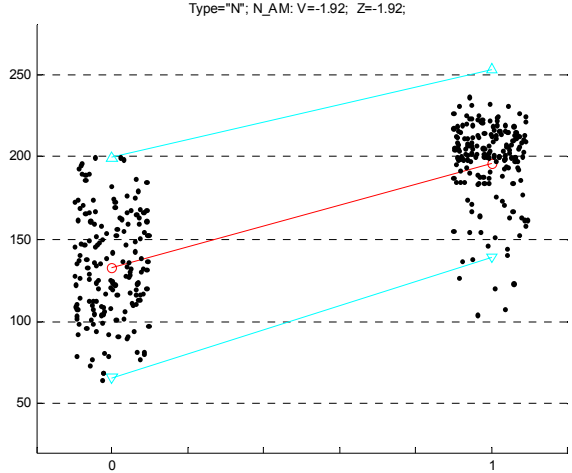




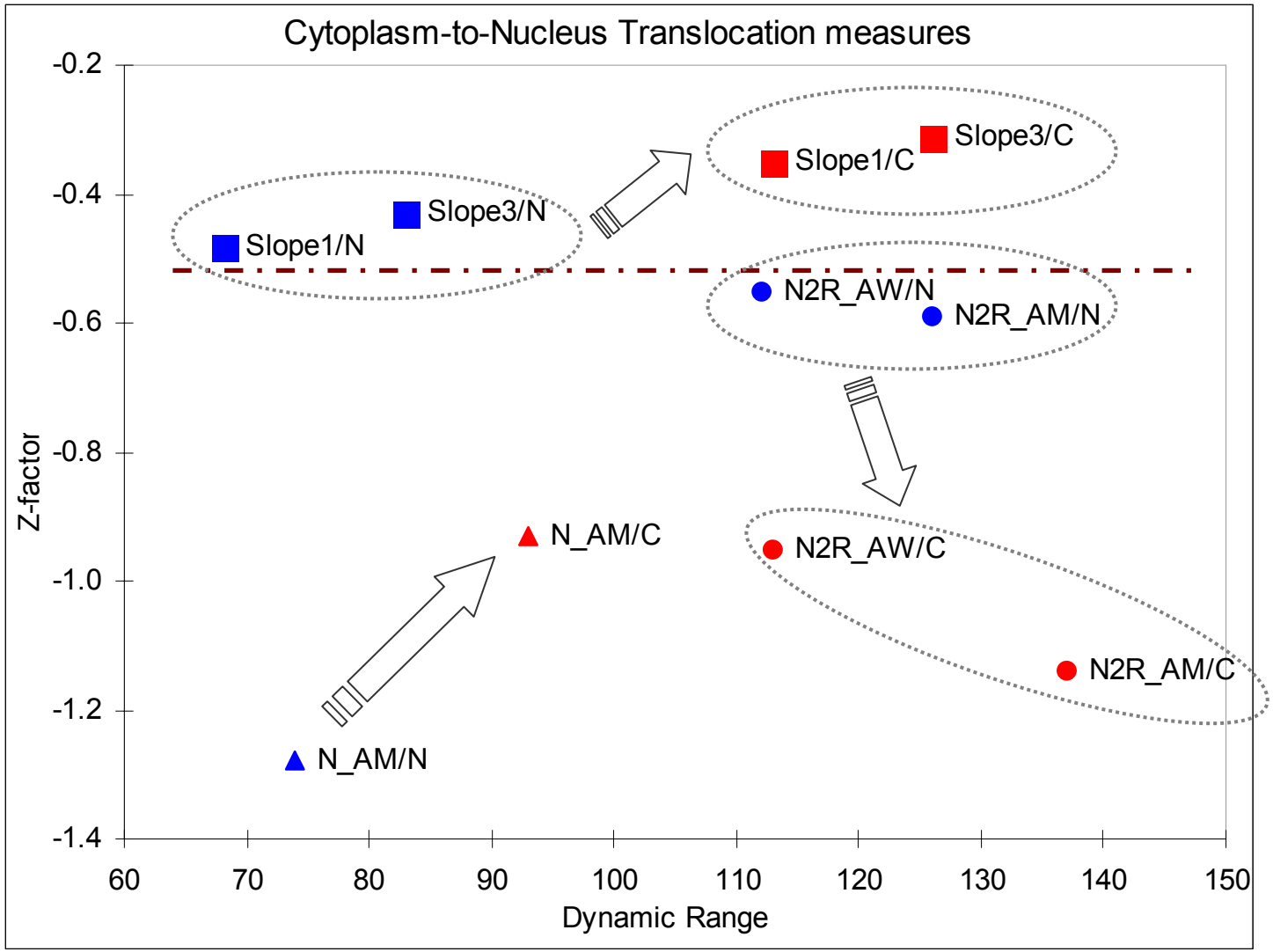
Camera







Cytoplasm to Nucleus Translocation Measures in the space of Z-factor and Dynamic Range



Red – Confocal (/C)

Blue – Non-confocal (/N)

– Slope measures

– Ring measures

– Nuclear measure

Conclusions

1. Slope measures are better than other measures regardless of imaging mode (confocal or widefield)
2. For slope measures confocal is better than widefield.
3. For ring measures confocal is worse than widefield.